COCCIDIOIDAL GRANULOMA: A CLASSIFICATION OF THE CAUSATIVE AGENT, COCCIDIOIDES IMMITIS[1]

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History

The purpose of this paper, as the title indicates, is to attempt a proper determination in the classification of fungi of the agent responsible for the disease clinically known by various names, as "blastomycosis" (Montgomery and Ormsby, '08), "oidiomycosis" (Giltner, '18, Ricketts, '01), "protozoic dermatitis" (Montgomery, '00), "dermatitis coccidioides" (Montgomery, Ryfkogel and Morrow, '03, Wolbach, '04), "blastomycetic dermatitis," "coccidioidal granuloma," and the "California disease." Of this list, coccidioidal granuloma has the most widespread acceptance, and in all references in this paper to the pathologic, clinical, or other diagnostic features of the fungus, this term will be used.

Since the report of the first case by Wernicke ('92) there have been 286 cases recorded, and with the one involved in this paper the total now is 287. The disease has received a great deal of attention because of its mode of infection, complicated diagnosis, lack of definitely demonstrable prophylactic measures, great percentage of fatality, peculiar pathogenic abilities, and last, but not least, the indefinite classification of the fungus itself.

In order to present a clearer perspective of the field, a résumé of the noteworthy points involved would be in order.

As stated above, the first case was reported by Wernicke ('92) from Buenos Aires. Clinically, the case was diagnosed as "mycosis fungoides." During the examination spherical organisms resembling protozoa were found in the lesions, and the cause of the disease was considered due to these bodies. Later reports showed that the patient had died of a general infection.

The second case was reported by Rixford in a brief note in the 'Occidental Medical Times' of 1894, later published with Gilchrist in 'Johns Hopkins Hosp. Repts.' ('96). Six months later in the same year, in conjunction with Thorne, he reported another

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case in the latter journal. In these two cases, the round organisms were seen in the smears made from the lesions, especially the purulent exudate. Various stages in the development were observed, some smears being filled with difficultly stained, irregular masses of material and some with a highly refractile, clear, non-staining capsule surrounding a group of spores formed endogenously, and stages between the two.

Pathologically, the condition was perplexing inasmuch as it simulated tuberculosis in all its clinical entities. Histological sections showed giant cell formation in the tissue with several of these round growths present.

Treatment with potassium iodide given internally in maximum doses, mercury in the form of protiodide, Fowler's solution with arsenic in gradually increasing doses up to tolerance, gave no beneficial prophylaxis. The various local antiseptics, as iodine, carbolic acid, bromine, bichloride of mercury, and several others, were applied externally, and some were injected into the lesion hypodermically but with no apparent relief. Both patients died.

In 1894 there were only two men in San Francisco who were equipped to do any bacteriological work, S. M. Mouser and Douglas W. Montgomery. The latter was called in on these cases, but could reach no definite conclusion as to the nature of these organisms. The consensus of opinion seemed to point to their being protozoa because of their resemblance to coccidia which cause the disease in rabbits and fowl known as "coccidiosis."

To determine the organism, some material was sent to W. H. Welch, of Johns Hopkins. Expressing doubt as to their protozoological nature, he turned the cultures over to T. C. Gilchrist, of the dermatology department, who had been working on blastomycosis, the lesion of which simulated those of the two cases noticed by Rixford and Thorne. Being unable, in turn, to determine the peculiar growths, he called in Stiles of Washington, an eminent medical zoologist and authority on protozoa. Perplexed as to this puzzling situation, Stiles named them "coccidioides" because of their similarity to the above-mentioned coccidia. Thus the organism from the first case he called *Coccidioides immitis* and that from the second, *Coccidioides pyogenes*.

Following this work, in which an appeal was made for reports of similar cases, there was nothing additional until Posadas ('00) reported a case and Ophüls and Moffit, in conjunction with Ash (Phila. Med. Jour. 5: 1471, June 30, 1900), in the San Francisco Hospital, reported another. The three latter authors were able to show that on artificial substrates the organism grew out into long mycelia, like a typical fungus of the ordinary mold group. They could thus demonstrate that there were two dissimilar life-cycles present: one in the body of the individual having the spherical forms noted previously, and the other being the filamentous growth present on cultural media. It must be added, however, that Montgomery ('00) had formerly noticed the mold-like development on the cultures he had studied, but, believing them contaminations, had discarded the growths.

From this time on, various attempts were made to study the life history of the fungus, and of these the work of S. B. Wolbach ('04) is outstanding. For this particular point in the paper, however, it suffices to say that the organism has been sufficiently studied and investigated clinically, to arrive at a clearer understanding as to its recognition. There is still much work to be done as to its various other phases.

Etiology and Symptomatology of Coccidioidal granuloma

Coccidioidal granuloma, as a disease, belongs in the class of infectious granulomas. Its course may be exceedingly acute and end fatally in a few weeks, or the agony may be prolonged and a period of several years may elapse before death. Then again, the time may be long and drawn out, with slight pain, as is apparent in the case to be mentioned here. Between the two extremes of time, however, there is a sub-acute type which is neither rapidly fatal nor unduly prolonged in its progress, but which is characterized by a definite and positive tendency to widespread dissemination in the patient, with remissions and relapses, and in which the sufferer lives from a period of six months to two years after infection.

There are many clinical types involved in the disease, and in 1905 Ophüls classified three:

1. Primary cutaneous lesions and later generalization;
2. Primary pulmonary lesions and later generalization, but no skin lesions;

3. Primary pulmonary lesions and secondary subcutaneous lesions.

Many years later, Jacobson ('30) added three other clinical types, and with the addition of two more reported in the literature the total is eight:

4. Primary pelvic involvement without any skin lesions;
5. Primary meningeal or spinal cord involvement without any cutaneous or other skin lesions;
6. Primary involvement of the bones with secondary skin lesions;
7. Primary joint lesions;
8. Primary subcutaneous lesions.

In the past, much attention has been given to the cutaneous and subcutaneous types of the disease, due to the fact that the greater number of cases recognizable were of those kinds and hence of interest to the dermatologists. As a result, the literature comprises numerous papers on these various conditions with an intensive study of them. However, in the course of time, when other clinicians became interested because of the spread and fatality of the malady, it was noticed that pulmonary infection was very outstanding and more generalized. Ahlfeldt ('26) was able to show experimentally that both pulmonary and cutaneous infections may take place.

Numerous workers have demonstrated the entrance of the organism through an abrasion or some wound in the skin. Then, because of its frequent presence in a hot climate, such as is found in parts of California, particularly the San Joaquin Valley, the pulmonary type of infection is common, through the inhalation of the spores. It is very probable that such clinical types as mentioned previously, involving the meningeal, subcutaneous and joint lesions, in addition to the primary pulmonary and bone lesions, are primarily lung infections. This involvement may not be noticeable at first, but autopsy reports usually show positive results in these cases.

The disease is protean in its clinical manifestations, with the result that it resembles very closely various other infections, particularly tuberculosis. Its multiform clinical entities may simulate, through a metastatic action on the part of the fungus, proliferating and suppurating processes, especially in the verrucous-like dermic lesions, as in the following: (a) The dermic lesions, which are nodular and ulcerative, in the form of painless, deep-seated, pinkish to dusky-red ulcers which become necrotic and sluggish, or may develop papillomatous growths, resembling epitheliomas, verrucas, tuberculosis in its various forms, syphilis, blastomycosis at times, and even sporotrichosis; (b) The subcutaneous coccidioidal involvement having three types of lesions —the flaccid tumor, the abscess, and the gummatous varieties may imitate cold abscesses or tumors as the names imply; (c) Pulmonary infections are usually diagnosed as pulmonary tuberculosis, but in many cases the correct diagnosis was determined post-mortem. Cases reported by Jacobson ('30), Montgomery ('00), Brown ('13), Hirsch ('23), and Taylor ('23) have given this information; (d) The osseous type of the disease has been confused with bone tuberculosis, osteomyelitis, or arthritis (Gardner, '04, Bowman, '19, Jacobson, '30). Hammack and Lacey ('24) found that twenty-one of twenty-three cases of generalized coccidioidal granuloma showed involvement of the bone. There is no roentgenological method of differentiating the disease from tuberculosis of the bone. Taylor ('23) suggested that "when bone destruction is particularly fulminating and when a proliferative process occurs along with the destruction, the diagnosis leans toward coccidioides rather than tuberculosis"; (e) Involvement of the meninges and the spinal cord usually requires a differential diagnosis from tuberculous meningitis, epidemic meningitis, and tumors of the spinal cord (Morris, '24, Rand, '30); (f) Gastro-intestinal disorders of coccidioidal granuloma require a thorough examination for the removal of the diagnosis of typhoid, as shown by Bowles ('12) and Carson and Cummins ('13). Cases reported along this line suggested very much the course usually taken by typical typhoid, and not until secondary cutaneous lesions had developed was an accurate diagnosis made; (g) Involvement of the lymph nodes usually suggests lymphatic leukemia, Hodgkin's disease, and lymphosarcoma. Ragle ('29) reported a case which early in its course resembled Hodgkin's disease and not until the cutaneous lesions appeared was the correct diagnosis given.

It would seem, therefore, that the diagnosis of coccidioidal granuloma is a difficult problem. Because of its close clinical association with tuberculosis, in order to establish the correct diagnosis it is necessary in every case to find the organism, in pus from cutaneous lesions or sinuses, in sputa, or in tissue from autopsy or biopsy material. Histopathological work does not prove anything unless the organism is seen. Frequently the lesions of coccidioidal granuloma show chronic inflammation without caseation, and some lesions may be purulent while others show the abundant necrosis typical of tuberculosis. At times, all these conditions may be present in the same section of tissue, so that it is essential that the fungus be sought. Single, negative, microscopic or cultural observations do not constitute a definite case against coccidioidal infection, for often in the presence of secondary pyogenic bacterial invaders repeated smears or cultures may be necessary before the organism can be found. Furthermore, inoculation in guinea pigs may be essential, and it is advised on all occasions.

Where the infection is of the primary visceral type with no opportunity for the study of either the tissue or the pus, the cutaneous allergic test has been applied. This consists of an intradermal injection of specific antigen obtained from a bouillon culture grown at 37° C. The filtrate of the culture, through a Berkefeld filter, contains the toxin which Jacobson ('28) claims to be an exotoxin. The principle of this reaction is that when the filtrate is injected cutaneously, a characteristic inflammatory reaction of the skin around the site of inoculation takes place in persons infected with the coccidioidal organism, whereas there is no toxic reaction in those free from the disease. This allergic cutaneous reaction may be due to an acquired cellular hypersensitiveness of the patient to the extracellular products of the organism, and the local manifestation is analogous to the tuberculin and luetin reactions. However, this method is suggested with caution and should be used only by trained investigators.

Positive tests have been reported by Davis ('24), Hirsch and Benson ('27), Jacobson ('28), and Chipman and Templeton ('30), while Cooke ('15) obtained negative results.

In addition to the types of involvement mentioned, autopsy reports reveal an enormous amount of pathological changes. These infectious conditions show the disseminating ability of the fungus. Of the great number of cases reported, the following conditions have been frequently observed:

Subcutaneous abscesses;
Lesions involving tissues of the head and neck;
Lesions involving the skin;
Meningitis and small granulomatous lesions within the brain substance;
Osteomyelitis of cranial bones with epidural or subcutaneous abscesses;
Oesophageal ulcer;
Lesions involving shoulder girdle or upper extremities;
Lesions of the thorax or its viscera;
Lesions involving the bony thorax (either ribs or sternum);
Miliary involvement of the lung;
Pneumonic consolidation simulating tuberculous pneumonia;
Fibrocaseous nodules without miliary involvement;
Caseation of hilar nodes;
Lesions involving the heart (pericarditis and endocarditis);
Lesions of the abdominal wall, spleen, liver, kidneys, pancreas, adrenals, iliac bones;
Lesions involving the whole genital apparatus, epididymus, and perirectal tissues;
Lesions of the bones of the pelvis or lower extremity;
Lesions involving the sacrum, both patellae;
Lesions in the region of the knee, and involving the ankles;
Erosion of the bodies of the vertebrae;
Involvement of the femoral, inguinal, retroperitoneal, mesenteric, lumbar, cervical, mediastinal, and peribronchial lymph nodes.

Agglutinins.—Immunological reactions have so far yielded unsatisfactory results. No agglutinins could be demonstrated by Cooke ('15), by Cummins and Sanders ('16) in experimentally infected animals, or by Davis ('24).

Precipitins.—Specific precipitins were demonstrated by Cooke ('15) in positive serum, in dilutions of 1 : 160, with an extract of dried cultures of the organism as a precipitinogen, but negative results were obtained with the same antigen and normal serum or when specific immune serum was tested with an antigen similarly prepared from a blastomycetic organism. Positive results were obtained by other workers (Chipman and Templeton, '30), but in lower dilutions, while Cummins and Sanders ('16) report negative precipitin reactions.

Complement-Fixation.—Complement-fixation has been demonstrated with high concentrations of antigen by Davis ('24) and Chipman and Templeton ('30), whereas Cooke ('15) and Cummins and Sanders ('16) obtained negative results, although it must be pointed out that Cooke used a saline emulsion.

Specific Soluble Substance.—Hirsch and Benson ('27) and Hirsch and D'Andrea ('27a, '27b, '30) were able to demonstrate the specific soluble substance mentioned previously under the allergic reaction. Prolonged electro-dialysis of the filtrate from broth cultures causes the separation of white floccules. The dried specific substance is a white powder, not destroyed by heating to 80° C. for thirty minutes, readily soluble in water, physiologically saline, dilute alkalies (N/10 NaOH) and dilute acids (N/10 HCl), contains about 3–4 per cent nitrogen, and on hydrolysis 20–40 per cent reducing sugar measured as dextrose.

Mortality.—Coccidioidal granuloma has caused a great deal of excitement because of its high rate of mortality. Starting with as high as 100 per cent fatality, the number has gradually fallen until present reports show it to be approximately 65 per cent. As yet, this is a rather high percentage for all cases of infection, for it is probable that many cases do not reach the literature, or there may be many unrecognized mild cases, as pointed out before. Some cases may even have spontaneous recovery or become dormant and hence not noticeable. Faulty diagnosis may also be given as a reason for too few reports.

Treatment.—No wholly successful treatment of coccidioidal granuloma has been found. Recovery may be spontaneous, or death may come on slowly. Roentgen rays, surgery, iodides, and intravenous injection of crystal violet, arsphenamenes and tartar emetic have generally been unsuccessful. Antimony and potassium tartrate (Guy and Jacobs, '27) have been reported as successful by Tomlinson and Bancroft ('28) on a medical student who had evidently contracted the disease while working on the organism. Jacobson ('27) used colloidal copper in conjunction with a special vaccine with encouraging results. Although his patients may be still alive, permanent cure is indefinite since remissions and relapses are apt to occur. In some cases, amputation of a limb has resulted in clinical cure.

Practically all races are affected, and of all the patients the greater number are males between the ages of 25 and 55. The higher percentage is in the agricultural class, the workers of the soil and its products.

Direct transmission from man to man or animal to animal has not been reported, although it is known that twenty-eight animals have had the disease. Besides, the organism has not until recently been isolated from the soil or vegetation where cases have occurred. *Coccidioides immitis* was obtained in cultures from the soil on the Delano Ranch in California where four cases had occurred among the Filipino working crew (Stewart and Meyer, '32).

Geographical Distribution.—The condition known as coccidioidal granuloma has a rather peculiar geographical distribution. Of the 286 cases reported prior to June 1, 1931, according to the bulletin issued by the California Department of Public Health ('31), 128 have been published, and a study of these cases shows that there is a decided concentration in California, in the San Joaquin Valley. Of this total number, 89.5 per cent or 254 cases originated in that state, the remaining cases having a widespread appearance as follows:[1]

South America	14	
Naples, Italy	2	
United States	16	
Arizona	2	
Colorado	3	
Illinois	2	
Kansas	1	
Missouri	1	(total now 2)
Nebraska	2	
Pennsylvania	1	
South Carolina	1	
Tennessee	1	
Texas	1	
Washington	1	

[1] Coccidioidal granuloma. State of Calif. Dept. Public Health, Spec. Bull. 57: 19. June, 1931.

As a result of its frequent presence in California, the term "California disease" has become the synonym of coccidioidal granuloma. It is hard to conceive how an organism which thrives so luxuriantly in animal tissues and which apparently finds the human body an excellent host and environment for its nutrition and propagation would willingly confine its activities to such large centers as Illinois, Pennsylvania, Missouri or Texas, to one or two persons, without manifesting any further evidence of its existence for a number of years. As mentioned previously, cases are occurring with rapid recovery or incorrect diagnosis, or the benign condition of many of the cases has not aroused enough interest in the attending physician to report the case. However, the disease has been made reportable, and it is hoped that all cases will be studied for future work.

Case Summary

The case involved in this report is worthy of note, since it is the second case, at the time of publication, known to occur in Missouri. However, due to certain factors, it is probable that the primary focus was California and not the above-named state.

In 1916, during his service in the Saint Louis City Hospital, Lipsitz, in connection with Lawson and Fessenden, reported a case of coccidioidal granuloma in the 'Journal of the American Medical Association.' Tuberculous broncho-pneumonia was at first given as the clinical diagnosis, but during the course of the disease numerous abscesses developed in the muscles, thus making it of a malignant nature. Death followed seven weeks after the onset of the symptoms. This patient had never been to California. This latter fact, coupled with the malignancy, made it an outstanding case.

For the second case, a brief history of which follows, the author wishes to express his gratitude to Dr. George Ives, of the Beaumont Clinic in Saint Louis, for the material and use of the data. The patient, a Saint Louis business man of about sixty years of age, visited California in August, 1927, and spent a short time in San Francisco and Los Angeles. Two years later, July, 1929, he developed a left-sided pleurisy with effusion, for which he was treated, and his recovery seemed complete. In April, 1931, approximately two years later, a mild arthritis of the right knee developed. During the following summer (1931), he took sun baths at Atlantic City, but to no avail.

In September, 1931, the patient consulted Dr. Klinefelter, who found the Wassermann test negative, and the Schilling and leucocyte counts to be normal. However, the joint contained a considerable amount of fluid, the removal of which gave the patient considerable relief, although temporary. There is no edema, redness of adjacent skin, or apparent increased temperature of the skin. The patient is healthy and has gained weight since diagnosis, and the evidence, except as shown above, gives no clue to a diseased person.

In contrast to the first case which was extremely malignant, this one has shown a very mild course, and it is noteworthy that these two extremes should occur in the same locality.

The joint fluid showed a light-yellow, turbid color and small masses of fibrin, with some blood and 8000 leucocytes, approximately 75 per cent of which were polymorphonuclears. Smears for bacteria were negative.

A guinea pig was inoculated with the sediment of the fluid and watched for a month, at the end of which time an indurated lesion developed at the site of inoculation and both inguinal lymph nodes became enlarged. The disease was first diagnosed as tuberculosis, but when the autopsy revealed no tubercle bacilli but did show acid-fast, imperfectly spherical bodies with granular centers the possibility of blastomycosis set in. It was from this pig that the author obtained the culture for study.

Technique

To determine the morphology and to obtain cell measurements, mycelium was mounted in hanging-drop cultures and observed. In this manner, the various steps in mycelial development were also watched.

For cellular detail, transfers were made to a solution of glycerine (Merck C. P.) and crystal violet (1 per cent aqueous). The material was cleared and stained at the same time by this method, allowing for as rapid a diagnosis as is needed where many cultures are to be examined. The time necessary for the process varies from 20 to 40 minutes; usually 30 minutes is sufficient, although in some cases only 10 or 15 minutes are needed.

Flemming's weak-killing and fixing solution was also used, being added directly to a tube of the culture. However, since the technique involved is long and the results are not materially different, it being necessary to stain and clear the material, steps which are extensive and time-taking, the former process was used almost exclusively.

Description

The fungus known as *Coccidioides immitis* has been shown by Ophüls and Moffit in 1900 to have two life-cycles: one in the host or tissue where it appears as a double-contoured cell varying from 5 to 60 μ in diameter and showing various amounts of granulation; the other on artificial culture media where it assumes a mold-like growth, with an intertwining network of mycelium composed of variously shaped, septate hyphae.

Inasmuch as the culture used in this paper was a mycelial growth on artificial media, the author had to content himself with an examination of the slides of tissue showing the round spheres, and wait for the development of the latter in anaerobic cultures. The forms observed, however, were typical of the classical cells.

In tissue, as stated above, the fungus grows and reproduces by endosporulation, a process which has caused a great deal of trouble in the classification of the organism. Numerous workers have made a study of this particular phase of the history of *Coccidioides immitis*, and Wernicke ('92) and Rixford and Gilchrist ('96), three of the first workers, gave the earliest description of the organism in tissue, the latter making the following statement:

"The parasite, when fully grown, is enveloped in a distinct, double-contoured capsule, and then appears as an almost perfectly spherical organism. . . . These forms vary from 15–17 microns in diameter and consist of a thick, well-developed, spherical capsule, which can be deeply stained. Between the capsule and the contents is a clear, refractive layer which usually does not stain or is stained with difficulty. This clear zone appears homogeneous and structureless; it varies in thickness from 2 to 3 microns, but is hardly discernible when the organism is undergoing sporulation. The protoplasm surrounded by the clear layer stains very readily; it is for the most part finely granular, but contains also not a few scattered, coarse granules, sometimes arranged around the periphery, at other times entering into the formation of a network. . . . The 'protozoa' present in this case are reproduced by sporulation. . . . The number of sporozoites which are finally developed from one organism varies, but is usually very large. During the process of sporulation, the capsule can be observed to become thinner and thinner until it consists only of a faint, but well-defined membrane which finally bursts. Just before this bursting stage, the organism changes its shape and assumes an oval form. The rupture takes place at one side or at both sides of the ovoid. One photomicrograph shows a number of fine prickles extending out from the capsule, especially at the sides."

It will be noticed that Gilchrist refers to the cells as 'protozoa.' This was the opinion held by several investigators until the mycological relationship was established in 1900.

Apparently, this description has been accepted, at least for the greater part, for we find that Ahlfeldt ('29) remarks: "We have found these prickles in several sections, and they are found only in adult organisms when they are ready to liberate young forms. We are able to confirm this method of sporulation, but think that the sporulating stage assumes an elliptical rather than an oval form."

Wolbach ('04) made a careful study of the life-cycle of *Coccidioides immitis* in tissue, pus, and sputum, and found that the contents of the cells may be "finely granular, almost homogeneous, or coarsely granular, reticulated, or vacuolated." The capsule may show radial striations, although usually homogeneous, a structure similar to that observed by Gilchrist and Ahlfeldt. He found that only the homogeneous cells segmented. The process begins with a division of the protoplasm peripherally, which extends inwards, forming many segments which are separated from one another by clear spaces. This is analogous to the mode of segmentation in a fertilized frog's egg, only here the divisions are simultaneous and not arranged in a series. The hyaline membrane then develops around the future spores. By the thinning out process of the capsule, the spores are liberated and held in groups by the growth inwards of the inflammatory tissue. That is apparently why so many of these encapsulated asci may be found in masses and not spread out evenly.

Inasmuch as the spores are non-motile, it is very likely that their spread in the tissue does not take place until the process of necrosis sets in, bringing about a moist condition. In this state of the lesion, the fungus finds its way into the lymphatic or blood streams and sets up endosporulation wherever held up, an act similar to metastasis.

Since the discovery that *Coccidioides immitis* had mold-like entities, several investigators have been able to demonstrate the change from the sphere to the mycelial growth and vice-versa. When placed on artificial media, the spheres send out thin, branched, septate hyphae. This phenomenon has been observed by Wolbach ('04) and MacNeal and Taylor ('14). The number of filaments so developing is indefinite, and, if we accept Wolbach's report, come apparently from the capsule, since he states that "the protoplasm meanwhile may remain shrunken within the capsule, and without demonstrable connection with the growing filaments." This occurrence, however, is opposed to that found by the latter authors who make the following statement:

"The capsule was penetrated at several points by blunt, protoplasmic outgrowths from the interior protoplasm. The rapidity of development of these shoots in some instances was such as to give the impression of ameboid movement at the growing tips. The shoots, at first naked cylinders of granular protoplasm, soon produced a definite, more hyaline wall about them, branched abundantly and irregularly and developed septa. After several hours, ovoid bodies of more homogeneous structure were numerous within many of the cells. After 24 hours, the circular colony had attained a diameter of one millimeter, and was made up of branched, interlacing, septate threads from 2 to 8 microns in diameter, with the old capsule of the original sphere as the center."

This latter quotation is in accord with observations made by the author on these endogenous, sporulating asci obtained in anaerobic cultures and grown on nutrient agar. The phenomenon is rather apparent, since an ascus which had fully matured and ruptured allowed the spores to spread out and develop (pl. 25, figs. 1–4). It will be seen that the spore elongates, becomes branched, with cross-walls, and the mycelial characteristics of the common molds, the varied hyphae measuring from 1 to 1½ μ in width, become abundant.

As the hyphae develop, they become wider, depending on the media on which grown, but averaging 2 to 2½ μ, being as much as 4 μ in width on some substrates. The wall becomes thicker and swellings arise on the filaments, these latter tending to form the racquet mycelium characteristic of *Coccidioides immitis* and suggestive of *Endomyces capsulatus* and the *Trichophytons* (figs. 5–12). With this increase in size the cytological detail becomes complex, and a number of deep-staining, round masses are seen which represent the nuclei and future spores. Hyphal enlargements, measuring approximately $5 \times 11\ \mu$, become abundant as the culture gets older. These enlargements have large amounts of densely stained material representing the beginning of chlamydospore development (figs. 7–8).

With greater age there is an increased growth in width of the hyphae, the cell walls become thicker, and then there is a resorption of cytoplasm (fig. 12), representing the beginning of arthrospore formation seen in figs. 13 and 14, and the development of spores. The cell membrane becomes clear, showing a hyaline space between the inner and outer walls. The cell contents change from an irregular, granular mass to a smooth, homogeneous substance, followed by the formation of spores with a clear space around each. In the meantime, however, the structure is changing from a rather rectangular cell to a sphere. The cytoplasm, representing the connecting link between each of the arthrospores, now becoming chlamydospores and then asci, has changed from a smooth cell to a very clear membrane which either becomes absorbed by the adjacent cell wall, or completely disintegrates. From observations, it is believed that the latter of the two is the more probable. In any case the cells tend to become round (figs. 18 and 19) and may form chains or be single. On artificial media, the tendency is for the cells to remain in chains when young, and to become large and single when the culture is older.

It usually requires about 8 to 10 days for these large spheres or asci to form in a definite amount on an agar substrate, this being in accordance with the work of Ahlfeldt ('29), and several others (Ophüls, '05, Wolbach, '04, MacNeal and Taylor, '14). At the end of several weeks, the colony has become fluffy and aerial hyphae are in abundance, the asci being numerous and loose. The medium assumes a brownish color imparted to it by the mycelium which has turned to a smoky brown.

Several workers have attempted to connect both phases in the life-cycle of *Coccidioides*. Wolbach ('04) and Ophüls ('05) found similar results in that a sphere developed from a segment of the mycelium, observations compatible with those made by the author in anaerobic cultures. The work of these men consisted of injecting mycelium into animals and watching the growth. In tissue and in body fluid, within the individual, chains are very few and the single endosporulating bodies are large. These organisms then go through the cycle described.

Cultural Descriptions

The culture furnished in this study was growing on a Sabouraud's agar slant. Colonies were many and small, spreading over the surface of the tube. Transfers were made to a series of media ranging from the more highly concentrated hydrogen ions to the less concentrated, on the pH scale roughly from 4.00 to 7.53. All cultures were grown at 25° C.

Because of its presence in a human lesion, it was thought best to use media which contained protein as a source for nitrogen. It was found, however, that the organism grew quite well on the simplest carbohydrate media as well as on agar nutrients. This is in accord with the work of Ophüls ('05) and of others, especially that of Bump ('25). In general, it was found that the organism grew quite well on a wide pH range, but the number of reproductive bodies was greatest on protein media. Inasmuch as the fungus can be identified by its microscopic morphology on various media, no particular attention was paid to its fermentative abilities at that time (Proescher, Ryan, and Krueger, '26).

Following are descriptions of colonies on several of the media on which determinations were made. These are arranged in the order of decreasing concentration of hydrogen ions.

Raulin's Solution (pH 4.15).—Colony white, filamentous, growing in large flakes partly submerged in the medium. Hyphae 1½ μ in width, long, few cross-walls, with swollen portions 2½ × 6 μ. Older hyphae show few chlamydospores and arthrospores.

Richard's Solution (pH 4.36).—Growth similar to that on Raulin's solution, mycelial growth being more abundant. Colony gray-white in color and in large filamentous flakes. Hyphae very thin, ½ to 1 μ in width, showing hyphal swellings and a few chlamydospores.

Czapek's Agar (pH 4.43).—Mycelium white, loose, cottony in growth. Hyphae 1½ to 2 μ wide, with chlamydospores and swellings suggestive of the racquet mycelium of *Trichophyton*.

Malt Extract Agar (pH 5.20).—Colony creamy-white in color, becoming brown after several weeks. Growth loose and cottony, thick at the inoculum, forming concentric circles, and attaining a diameter of 9 cm. in 5 weeks. Hyphal swellings present, round, thick-walled chlamydospores in abundance, suggestive of the "*endoconidies*" of Vuillemin ('99), and the "*globules internes*" of Salvat and Fontoynont ('22). These measure from 4 to 7 μ in the culture.

Sabouraud's Agar (pH 5.60).—Growth rapid, cream-colored when young, becoming light brown with age. Mycelium thick, cottony, reaching a diameter of 8½ cm. in 5 weeks. Hyphae long, growing up on the side of the tube, attaining a width of 2½ μ in mature stage. Abundance of chlamydospores and arthrospores in older colonies, having various sizes and shapes. Hyphal swellings also present and numerous.

Oat-Meal Agar (pH 5.80).—Growth very loose and cottony, color white, not turning brown with age. Hyphal measurements similar to that on Sabouraud's agar.

Corn-Meal Agar (pH 6.00).—Growth loose and cottony, similar to that on oat-meal agar, reaching a diameter of 8 cm. in 5 weeks. Mycelium loose around inoculum, followed by a heavier growth. Hyphae 1½ μ in width, forming swellings 2½ to 3½ μ in width and 4½ μ in length to the normal hypha. Arthrosporous formation abundant in the older colonies.

Gelatin (pH 6.70).—Medium liquefied. Growth cone-shaped, with loose, cottony, branching, septate mycelium 2 μ in width, penetrating to a depth of 2.4 cm. in 5 weeks. Mycelium as on other media.

Beef Extract Agar (pH 6.81).—Growth loose and cottony, spread over surface of plate, reaching a diameter of 9 cm. at the end of 5 weeks. Color white, becoming brown with age. Abundance of thick-walled cells. Measurements same as on Sabouraud's agar.

Glycerine Agar (pH 7.00).—This medium consists of beef-extract agar with 6 per cent glycerine. Growth most favorable on this agar, with an abundance of spores (arthro- and chlamydo-), at first thick at inoculum with a thinner area surrounding it, that being encircled by a thick elevated mass; size of colony reaching a diameter of 3 cm. in 7 days and 8 cm. in 5 weeks. Hyphae thicker than on other media, having a width of 2½ to 3 μ, with the swollen parts attaining a width of 7 μ.

Eosine-Methylene-Blue Agar (Product of the Digestive Ferments Co., pH 7.00).—This medium was used merely as a part of a routine. Growth characteristic of the group, similar to that on Sabouraud's agar, with a diameter of 8 cm. in 5 weeks. Hyphae 2 μ wide, septate and branching, with the characteristic swellings and the formation of chlamydospores in the older colonies. Colony assumes a pink color which spreads over the mycelium, completely halting growth after 6 weeks.

Nutrient Agar (Product of the Digestive Ferments Co., pH 7.27).—Growth similar to that on beef-extract agar. Diameter of colony 9 cm. at end of 5 weeks.

Endo's Medium (pH 7.53).—Growth very slow, dye of medium being absorbed by inoculum, with growth stopping after 2 weeks.

Anaerobic Media.—Liborius' method of anaerobic cultivation was used.[2] Growth present, but poor, as contrasted with that in aerobic circumstances. The organism thus shows a facultative aerobic condition.

Summary of Cultural Work

The organism involved in the case mentioned previously was found to be characteristic of the fungus, *Coccidioides immitis*, based on the following properties, both culturally and morphologically, as seen in the experimental work:

1. Ability to grow on a wide variety of media as indicated.
2. Occurrence on a wide range of pH.
3. Color changes to a light brown with age.
4. Characteristic flaky growth on liquid media.
5. Condition of facultative aerobiosis.
6. Branching, septate mycelium.
7. Peculiar hyphal swellings.
8. Characteristic mycelial measurements.
9. Formation of the chlamydospores.
10. Formation of ascogenous cells.
12. Endogenous spore formation in anaerobic conditions.

[2] Method taken from Hiss-Zinsser, Textbook of Bacteriology, p. 146. New York, 1929.

Discussion

As many as are the terms applied to the disease, so varied are the names that have been used in designating the organism. If we go back to the time when the fungus was first found (Wernicke, '92), we would notice that it was called a protozoon. In fact, so great was the belief in its zoological affinity that when, some time later, two more cases were observed (Rixford and Gilchrist, '96) the medical zoologist, Dr. Stiles, was called in to render a diagnosis. He called the organism of the second case *Coccidioides immitis*, because of its resemblance to a protozoon, and that of the third case *Coccidioides pyogenes*.

Several years later, in 1900, Ophüls and Moffit, in conjunction with Ash, found that the organism was similar to a mold, and gave it the name *Oidium coccidioides*, and referred it to the class Ascomycetes. It was also termed *Oidium protozoides*.

Ricketts ('01) studied seventeen organisms and concluded that they belonged to the genus *Oidium*, distinguishing the following types as varieties: (1) Blastomycetoid or yeast-like; (2) Oidium-like; (3) Hyphomycetoid.

Verdun in 1907 prefers to call the fungus *Oidium immite*, while Brumpt ('27) insists on its classification as a hyphomycete, *Mycoderma immitis*, and to make matters more complicated, Castellani ('28) renames the genus *Blastomycoides*.

The classification of *Coccidioides immitis* has been very uncertain and the names applied to the organism very indefinite, with the result that no established taxonomic position has been assigned to it. There are those who assume that it is a hyphomycete, with definite hyphomycetous characters, but the position of these authors is very shaky as we shall see. On the other hand, many believe it to be an ascomycete, showing ascus formation and definite mycelial characteristics, and it is in this group that the classification here to be established is involved. To avoid any confusion, the California Department of Public Health has named it "fungus coccidioides," which makes an additional term to deal with.

An analysis of these terms will immediately eliminate several of them. In the first place, there is no evidence of budding in any of its forms. In the tissue, as described previously, spore formation occurs chiefly through endosporulation. In some cases, however, instances have been observed where the condition simulated budding very much, in that two spheres accidentally grew together with adjacent surfaces attached, but these cells had never become detached while young. Old cultures usually show a similar condition when two or several segments of the mycelium have never become separated by the disintegration of the cell structure between them (pl. 25, fig. 18). This phenomenon has led many to refer the organism to the genus *Oidium*, but since budding is absent from the life-cycle of *Coccidioides immitis*, it cannot be included in the group where this type of reproduction may occur.

Also, a glance at the development of mycelium and the formation of the arthrospores and then chlamydospores, producing what the author prefers to call asci, shows that they are not oidia. Thus the term blastomycete, as used in its literal sense, would be void here. Furthermore, the term *Mycoderma*, as defined by Brumpt, is equivalent to *Oidium*, and must be eliminated, since the fungus reproduces in the tissue by budding and never by endogenous spore formation. Brumpt now prefers to designate *Coccidioides immitis* as belonging to the Chytridiales of the Archimycetes. On examining the group, this view would seem a good deal more logical than the former conception, but the characteristic septate hyphae present in *Coccidioides* are lacking in the chytrids, hence that idea is amiss.

In Vuillemin's classification of the Fungi Imperfecti ('10), the groups are divided on the basis of their reproductive methods or on the kind and manner of spore formation. Thus in one of the orders, Thallosporales, the cells, thallospores, formed by the vegetative mycelium, are of a vegetative nature and not particularly suited for reproduction. Here we find accordingly two suborders: (1) the Blastosporineae which reproduce by blastospores, including the budding yeasts or yeast-like fungi, as *Monilia;* (2) the Arthrosporineae, the group reproducing by arthrospores. This latter group includes such forms as *Oidium lactis* and comprises the Actinomycetes. Two families are present: (1) the Mycodermaceae, with mycelium simulating that found in the first suborder, and; (2) the Nocardiaceae, the Actinomycetes.

Of the latter two categories mentioned above, the Mycodermaceae have some interest here, but, in the first place, the mycelium is monilia-like, and in the second, the arthrospores referred to in *Coccidioides* are not of that nature, *sensu stricto*, but represent an early stage in the formation of asci. Furthermore, the characteristics of *Oidium lactis* are not found in the above group. On closer study, one finds that the group classified by Vuillemin as Thallosporales has several features in common with those of the Ascomycetes, following Gaümann and Dodge ('28). As a family, however, the Mycodermaceae include too many budding forms, and that condition would eliminate the transfer of *Coccidioides* to that category.

Several writers relegate *Coccidioides* to a generic position in the Protomycetaceae (Fonseca et Arêa Leâo, '28, Basgal, '31), to which they have also transferred *Endogone*, a member of the Endogonaceae of the Zygomycetes. It must be said here, however, that these authors have dealt with the organism occurring in South America. Almeida ('28) has summarized the principle features, on a comparative basis, of the organism found in the United States and the one present in Brazil, which, if we were to accept as significant of the fungus of the latter country, should be considered as different from *Coccidioides immitis* only as a species. This Brazilian fungus is similar to that described by Posadas, and it has been named *C. brasiliensis* by Splendore in 1912, emend. F. Almeida, but the term *Posadasia esferiformis* Canton 1898 has been used to designate the fungus of that region. In view of present knowledge, however, *Posadasia* is in synonymy with *Coccidioides*, and because of priority, *esferiformis* should be used as a specific name instead of *brasiliensis*. The term *C. esferiformis* would thus constitute a species of the same genus, in the same family. In referring the genus to the Protomycetaceae, the characters of two diverse groups have been intermingled, without indicating any connecting links. Furthermore, an examination of the Protomycetaceae (Büren, '15) shows that sexuality is prominent. Although showing similarity in some degree in the mycelial characteristics, as the chlamydospores, intercalary cells and branching, phenomena which may be present in a great many other fungi, still, when we approach the essential point of differentiation, the reproductive process, the act of spore development and dissemination pursues a different course. In the Protomycetaceae, with increasing age, the spores develop as a sheath on the inner surface of the third layer which elongates, breaks through the outer two coverings, accumulates the spores in a mass, and then shoots the aggregation into space.

Following this description of the Protomycetaceae, Mazza and Parodi ('28) have established a new genus, *Pseudococcidioides*, with properties similar to those found in *Coccidioides*. Since the growth on agar is not very well defined no exact classification can be established for this organism, but inasmuch as the whole process suggests that found in other members of the Protomycetaceae, particularly in the mode of formation of the spores and the presence of vacuoles, a tentative position as a new genus in that family may be assumed.

A reference must also be made to the genus *Rhinosporidium* which was established as a fungus by Ashworth in 1923, and which has been associated with the above genera in the family Protomycetaceae. This organism forms an ascocarp with a diameter up to 0.8 mm. Within this structure there is a great number of asci which are called pansporoblasts or morulas, which are liberated by the rupture of the ascocarp, each measuring 6 μ and containing 4 to 16 spores. An encapsulation of the organism by mononuclear leucocytes follows and then the process of growth continues. Several attempts to grow the cells on media have given no results, hence the determination as a fungus and the classification among the Phycomycetes, after Ashworth ('23), more specifically among the Olpidiaceae of the Chytridiales as Brumpt prefers it, or in the Protomycetaceae (Fonseca, '28), must of necessity rest indefinite in view of the scanty criteria present.

With this brief discussion of the conceptions of the taxonomic position of *Coccidioides*, let us turn to a consideration of the phylogenetic possibilities.

In the first place, it would seem that we are dealing with a very old organism. Here we have a fungus which has become so adapted to its parasitized host that it is able to go through a whole life cycle without a change of habitat. Of course, several steps have been eliminated in the process, but when we realize that the end result is the same, *Coccidioides* must be considered of extreme senescence. A second proof rests on the fact that the sexual process has degenerated to the state where copulation is lost and parthenogamy prevails. A condition of this sort may be found in certain of the Hemiascomycetes where the mode of development simulates very much that of the Protomycetaceae, except that the former have copulation, either iso- or heterogamous with a definite number of spores, an advanced character, while the latter have the copulation of spores. The group constituting the former category, the Endomycetaceae, is significant in that it has been treated by many as having definite copulating forms. However, an examination of its phylogenetic relationships establishes two developmental series, that is, the isogamous, as found in *Eremascus fertilis*, and the heterogamous, as found in *Endomyces capsulatus*, or *E. Magnusii*. Both series may end in parthenogamy. This latter fact is of extreme significance here, because an association of these characters helps to establish the position of *Coccidioides*.

After due consideration of the characters presented by the members of the Hemiascomycetes and a study of the criteria involved, it appears to the author that the organism in question, namely, *Coccidioides immitis*, should be placed in the Endomycetales. Having a relationship to the Zygomycetes, on the one hand, and a semblance to the Taphrinales with the Protomycetaceae, on the other, *Coccidioides* constitutes a division comprising the affinities of both. Receiving several of its features from the Endomycetaceae and others from the Saccharomycetaceae and Protomycetaceae, makes it necessary, in view of such facts, to establish a new family, Coccidioideaceae, with *Coccidioides* as the principle genus, and to place that family in a position following the Endomycetaceae and preceding the Saccharomycetaceae. Such a division would represent the parthenogamous end of the copulation series. Although difficult to conclude definitely as to which of the two mentioned previously this fungus may belong, nevertheless, judging by the particular mode of formation of the asci, isogamy, in all probability, had prevailed.

The life cycle of *Coccidioides immitis* may be shown diagrammatically, as follows:

(a) in the tissue,

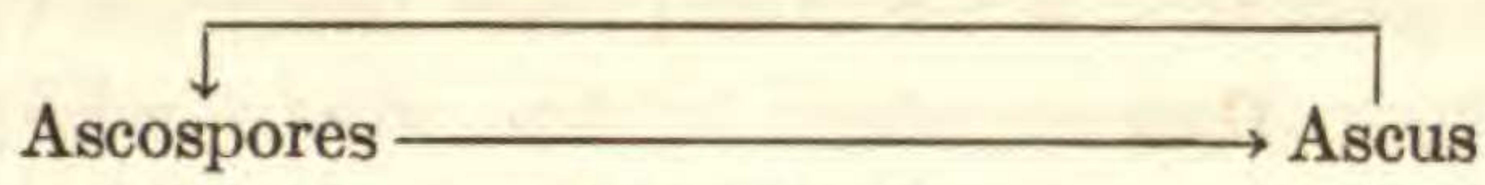

Diagram I.

(b) on artificial media,

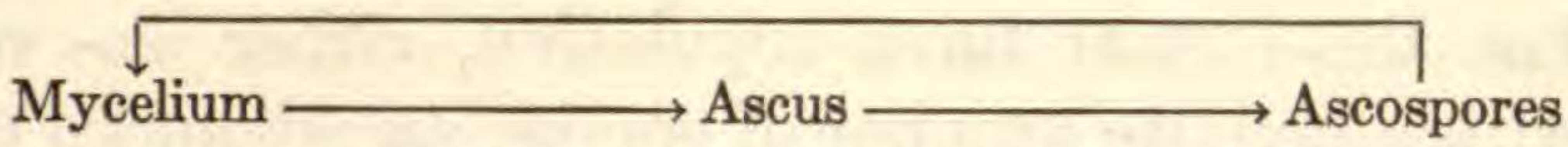

Diagram II

In the following diagram, the position of the new family in relation to the now-existing divisions may be easily noticed:

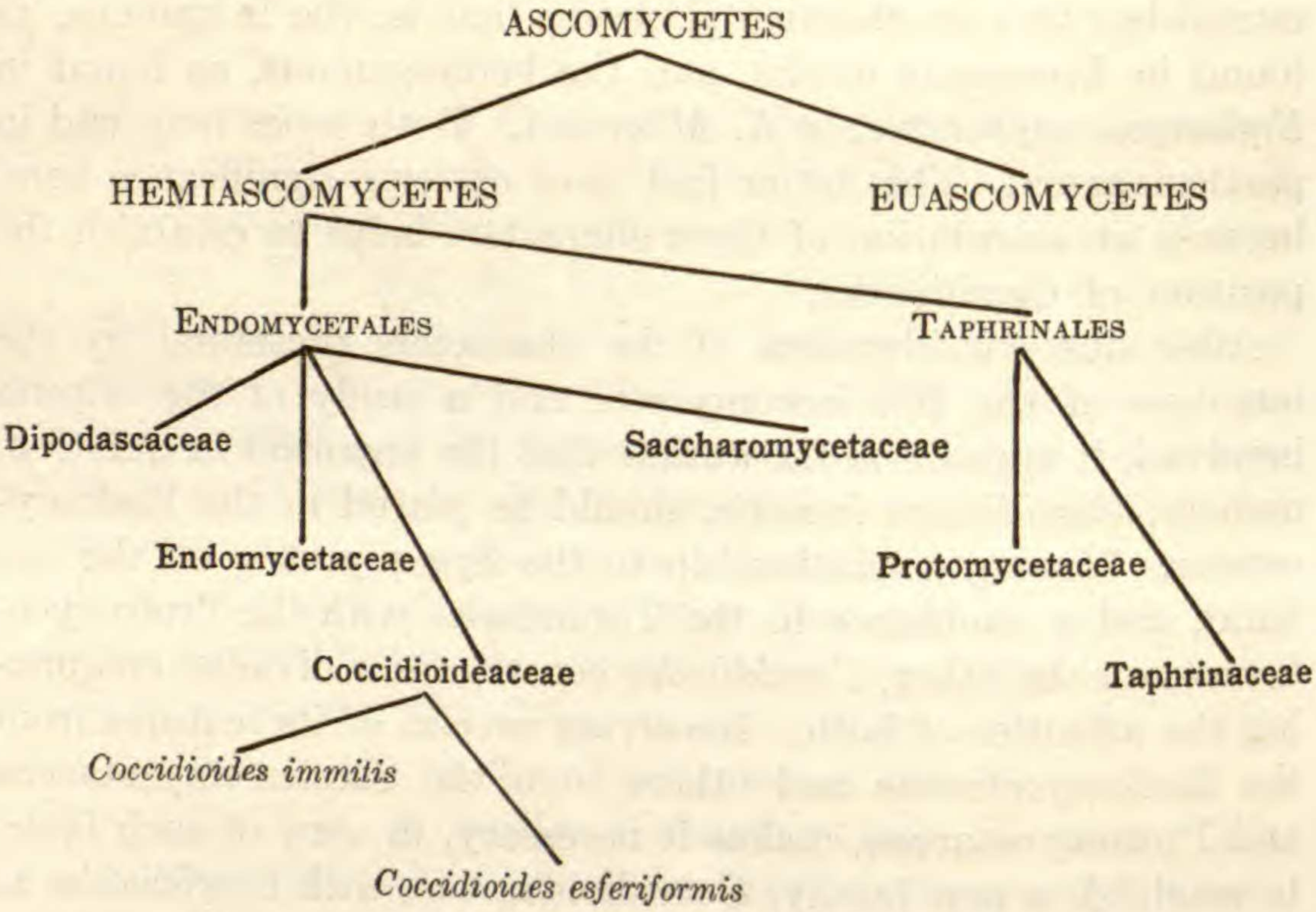

Diagram III

Coccidioideaceae Moore, new family.

This family differs from the others of the Endomycetales and Taphrinales in that the sexual development is completely lost and parthenogamy prevails; budding in any of its forms is lacking; an entire life-cycle is present in the parasitized host; endogenous, sporulating asci arise as a result of the disintegration of the intervening cells; and finally, the number and form of development of the spores is different.

Coccidioideaceae Moore, fam. nov.

Mycelium in culturis abundans sed in hospite fere deest; asci parthenogetici singuli ex cellulis hypharum degeneratione cellularum intermediarum orti, sporae in asco multae parvae ellipsoideae, sphericaeve.

Ab Endomycetaceis sexus gemmationisque carentia, sporarum in asco multitudine, a Saccharomycetaceis mycelii abundantia differt.

Coccidioides Stiles 1896.

Growth in tissue by endogenous spore formation with many spores, round to oval; on artificial media there is an abundance of mycelium varying with the type of agar used. Hyphae septate and branching, measuring from ½ to 4 μ in diameter, characteristic hyphal swellings and formation of terminal hypnospores. Budding entirely lacking, as well as copulation. Asci form from the hyphae through the differentiation of cellular material and the distintegration of intervening cell membranes, varying from 1 to 80 μ in diameter.

Coccidioides immitis Stiles 1896.

Development by endogenous spore formation in tissue, in asci varying from 4 to 80 μ in diameter; spores numerous, round to oval. Budding and copulation lacking. On agar, colony of abundant mycelium varying from a light pink when young to a smoky-brown with age. Hyphae septate and branching, ½ to 4 μ in diameter on different media. Hyphal swellings varying from 2½ to 7 μ in width and 5 to 12 μ in length. Chlamydospores abundant, 5 to 8 μ in diameter. Terminal hypnospores several, approximately 5 × 8 μ.

Coccidioides esferiformis (Canton 1898) Moore, n. comb.

Reproduction in tissue by dehiscence of spores from the interior through the membrane, until death of cell. Asci vary from 1 to 40 μ in diameter. Growth on media difficult, optimum at pH 7.4 after 20 days. Colony white to gray. Cultural characteristics otherwise similar to the above.

With the establishment of *Coccidioides esferiformis* as a species differing from *C. immitis*, the percentage of cases occurring in California prior to June 1, 1931, plus the present case, would rise to 93 per cent. It is probable that the cases found outside of California may have had some traffic with that State as is evident from the patient here examined, and if so it shows the fungus to be endemic to the above-named region.

Summary and Conclusions

1. The history of coccidioidal granuloma is given, with a review of the early diagnosis of the organism.

2. The etiology and symptomatology show various involvements and complications and eight clinical types.

3. The immunological reactions show an incompleteness in definite beneficial results.

4. The cases show a peculiar geographical distribution, with a localization of 93 per cent of the cases due to *Coccidioides immitis* in California, and the remainder spread throughout the country and 2 cases in Italy, while many cases due to *C. esferiformis* have occurred in South America.

5. A summary of the second case known to occur in Missouri is given, showing its extremely benign course as compared with that of the first.

6. The fungus is described in detail, showing its double life-cycle: one in the tissue as a sphere, and the other on artificial media as a mold-like growth. A study of the organism reveals its relationship to the group characteristic of the Endomycetales.

7. The organism was grown on various media, showing a wide range of properties typical of *C. immitis*.

8. The phylogeny and classification of the organism is discussed, with the result that a new family, Coccidioideaceae, is established and placed in a position between the Endomycetaceae and the Saccharomycetaceae, having the affinities of both. One genus and two species are at present recognized.

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Explanation of Plate

PLATE 25

Coccidioides immitis

Fig. 1. Young spore. × 960.
Fig. 2. Developing spore. × 960.
Figs. 3, 4. Young developing mycelium. × 960.
Fig. 5. Type of mycelium on nutrient agar. × 960.
Fig. 6. Type of mycelium on Sabouraud's agar. × 960.
Fig. 7. Portion of mycelium on glycerine agar. × 960.
Fig. 8. Probably a chlamydospore on Sabouraud's medium. × 1440.
Fig. 9. Terminal hypnospore. × 1440.
Fig. 10. Young hypha on glycerine agar. × 960.
Figs. 11, 12, 13, 14. Formation of arthrospores. × 960.
Fig. 15. Branching arthrospores. × 1440.
Figs. 16, 17. Old mycelium showing chlamydospores on malt extract agar. × 960.
Fig. 18. Formation of round chlamydospores from mycelium in anaerobic culture. × 960.
Fig. 19. Large, round ascogenous cell with endogenous spores. × 960.